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RESEARCH ARTICLE OPEN ACCESS

Value of hydroalcoholic treatment of rapeseed for oil extraction and protein enrichment☆

Morgane Citeau[1,*], Jennifer Regis[2], Patrick Carré[1] and Frédéric Fine[3]

[1] OLEAD, 11 rue Gaspard Monge, 33600 Pessac, France
[2] SAS Pivert, Parc technologique des rives de l'Oise, Rue les rives de l'Oise, 60280 Venette, France
[3] Terres Inovia, 11 rue Gaspard Monge, 33600 Pessac, France

Abstract – This study investigated alternative solvents: ethanol and isopropanol, to replace hexane and enhance the quality and value of oil and meal. Rapeseed oil extraction was carried out using ethanol (92 wt.% or 96 wt.%), isopropanol (84 wt.% or 88 wt.%) or hexane (as reference). Results show that hydroalcoholic extraction increased meal protein content by 13% compared to hexane extraction, but without significant influence of alcohol and water content. However, increasing water content improved glucosinolate extractability. Isopropanol 84 wt.% eliminated most glucosinolates from the seeds, decreasing glucosinolate concentration by 49–73% compared to meals extracted by the other alcohols.

Keywords: hexane / ethanol / isopropanol / rapeseed meal quality

Résumé – Intérêt des solvants hydro-alcooliques pour l'extraction de l'huile et l'enrichissement en protéines du tourteau de colza. L'étude a porté sur l'expérimentation de solvants alternatifs à l'hexane : éthanol et isopropanol à différents degrés d'alcool, visant à la fois une bonne capacité d'extraction de l'huile et l'amélioration de la qualité du tourteau grâce à l'augmentation de la concentration en protéines et à l'élimination de composants antinutritionnels type glucosinolates. L'extraction du colza a été réalisée par de l'éthanol à 92 % m/m et 96 % m/m d'alcool, de l'isopropanol à 84 % m/m et 88 % m/m d'alcool ou de l'hexane (solvant de référence). L'extraction hydro-alcoolique a augmenté la teneur en protéines du tourteau de 13 % par rapport à l'extraction hexane, mais sans influence significativement de la nature de l'alcool ou de sa teneur en eau. L'augmentation de la teneur en eau du solvant a par contre augmenté l'élimination des glucosinolates. En particulier, l'isopropanol 84 % m/m a été le plus efficace, permettant de diminuer la teneur en glucosinolates du tourteau de 49–73 % par rapport aux autres alcools.

Mots clés : hexane / éthanol / isopropanol / qualité du tourteau

1 Introduction

Vegetable protein production and use is a major agrifood-sector challenge for the future. On one hand, the ever-increasing global demand for animal proteins is driving co-demand for vegetable proteins as animal feed. On the other hand, the vegetable protein market for human consumption is booming, bringing with it a number of imperatives in terms of functional and nutritional quality.

Rapeseed meal is a source of protein, characterized by a well-balanced amino acid composition that offers potentially excellent nutritional value (Campbell *et al.*, 2016). Rapeseed meal comes from the oilseed crushing industry, where it is the main co-product, accounting for around 55% of rapeseed mass. After oil extraction, the rapeseed meal contains around 35–40% proteins, plus carbohydrates (30–35%), crude fiber (10–15%), minerals (5–10%), and up to 10% secondary plant metabolites (von der Haal *et al.*, 2014; Carré *et al.*, 2016). However, the current rapeseed oil production-line configurations limit the production of a digestible protein-rich meal (Grala *et al.*, 1994; Adem *et al.*, 2014; Mosenthin *et al.*, 2016). The presence of anti-nutritional components (fibers, glucosinolates, phytates and phenolics) and the loss of protein solubility during the crushing process hinders further value recovery (Bell, 1993; Quinsac *et al.*, 1994; Adem *et al.*, 2014). Rapeseed meal is used as animal feed, mostly for cattle and pigs, but it is less suitable for poultry or fish. Rapeseed meal is

☆ Contribution to the Topical Issue "Rapeseed / Colza"
*Correspondence: m.citeau@olead.fr

currently the world's second-leading source of vegetable protein, way behind soybean.

Increasing concerns for health, safety and environmental care have prompted to study alternative solvents in order to substitute hexane in oil extraction (Li *et al.*, 2014; Sicaire *et al.*, 2015; Baümler *et al.*, 2016; Breil *et al.*, 2017). Sicaire *et al.* (2015) and Breil *et al.* (2017) used physical properties of solvents (viscosity, boiling point, vaporization enthalpy and toxicity), theoretical solvent-lipids solubility and extraction experimentations in order to compare different solvents. They demonstrated that extraction efficiency varies depending on the lipid class or component (TAG, phospholids, tocopherols...), and the solvent choice depends on lipids composition of the meal.

Also, regarding the principles of Green Chemistry and Green Extraction (Chemat *et al.*, 2012), isopropanol and ethanol have many benefits as compared to other solvents. They are less toxic than other "green" solvents (Chemat *et al.*, 2012; Prat *et al.*, 2016), already authorized for food extraction by the European Directive 2009/32/EC, readily available and bio-sourced (Breil *et al.*, 2017; Perrier *et al.*, 2017). In term of energy, alcohols are not favorable in the conventional extraction process because of the energy required to evaporate solvent. However, extraction process can be adapted to the use of these solvents. For example, the distillation step of miscella can be replaced by cold demixing method to recover oil and solvent phase. This contributes to save energy and to lead to a more economically viable process (Carré *et al.*, 2018).

Isopropanol and ethanol are made more polar solvents than hexane, which has negative consequences on oil selectivity and miscibility (Sicaire *et al.*, 2015; Breil *et al.*, 2017). However, they are known to simultaneously solubilize oil and some non-lipid components of seeds, which helps to concentrate the proteins and detoxify the meal. According to Berot and Briffaud (1983), the extraction of de-hulled rapeseed flour by ethanol or isopropanol at 60 v/v% alcohol increased protein concentration from 53 to 61–63 g per 100 g of de-oiled dry matter and removed of 96–97% of polyphenols and more than 99% of glucosinolates, but not phytin phosphorus. Sinichi and Diosady (2014) used absolute isopropanol to extract de-hulled mustard seed, and showed that the extracted crude oil contained 15% of non-lipid dry matter with 12.8 g of carbohydrates and 2.2 g of nitrogenous matter per 100 g of dry crude oil. It could be deduced that the extraction yield of non-lipid components was around 6% of the initial mass of dry flour in the tested conditions.

Furthermore, studies have shown that the extraction efficiency of ethanol and isopropanol is hugely dependent on solvent water content and temperature. Thus, increasing water content in the solvent enhances the extraction of non-lipid components but reduces the efficiency of oil extraction. Fauduet *et al.* (1995) studied the influence of ethanol-water mixtures on the extraction of glucosinolates from rapeseed meal and showed that increasing water content in the solvent increased glucosinolates extraction yield: 94%, 40% and 14% of glucosinolates were removed by pure water, ethanol at 80 wt.% and ethanol at 97 wt.% alcohol, respectively. However, the use of pure water led to a 23% loss of the initial mass of proteins. Similarly, van Megen (1983) reported that decreasing alcohol content in ethanol (from 85 wt.% to 65 wt.% alcohol) increased the extraction yields of glucosinolates (from 49 to 92% at 60 °C) and dry matter (from 21 to 27% at 60 °C).

Table 1. Rapeseed composition.

Sample	Moisture content (g/100 g)	Total oil content (g/100 g dry matter)	Average protein content (g/100 g dry matter)	Glucosinolate content (μmol/g dry matter)
Rapeseed	4.8	49.5	19.2	17.2

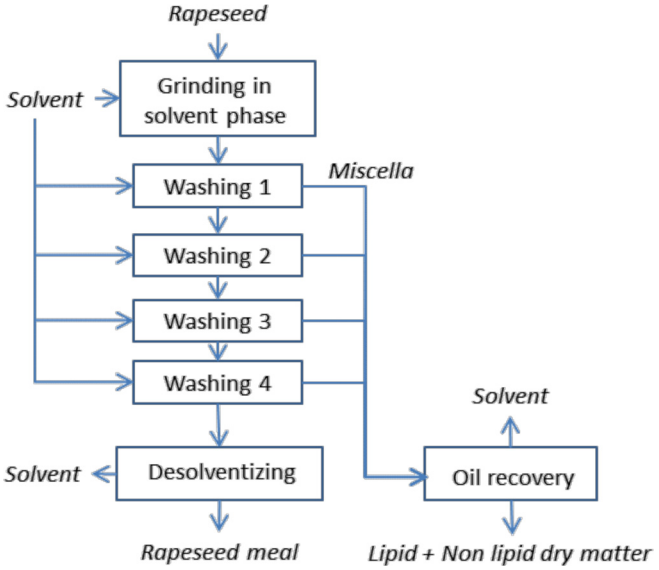

Fig. 1. Solvent extraction procedure.

Studies to date have mainly tested hydroalcoholic solvents with high water content and dealt with pre-defatted matter to evaluate the ability of solvents to concentrate proteins in the meal and/or detoxify the meal. The aim of this study was to find a non-hexane solvent with good oil extraction capacity without losing non-lipid extraction efficiency. This study investigated the influence of solvent type, *i.e.* ethanol and isopropanol at various water contents, on oil extraction and rapeseed meal quality.

2 Materials and methods

2.1 Materials

Rapeseed was purchased from Alliance Occitane (Toulouse, France). Table 1 reports the rapeseed composition.

Hexane, ethanol and isopropanol were purchased from Quaron (Cestas, France) and were "technical" grade.

2.2 Extraction procedure

The process flow diagram in Figure 1 presents the rapeseed extraction procedure. The seeds were first crushed by a Silverson L5M-A high-shear laboratory mixer in the solvent phase (4 min at 8400 rpm and 15 °C). The extraction experiments were then carried out in an agitated Nutsche filter (POPE

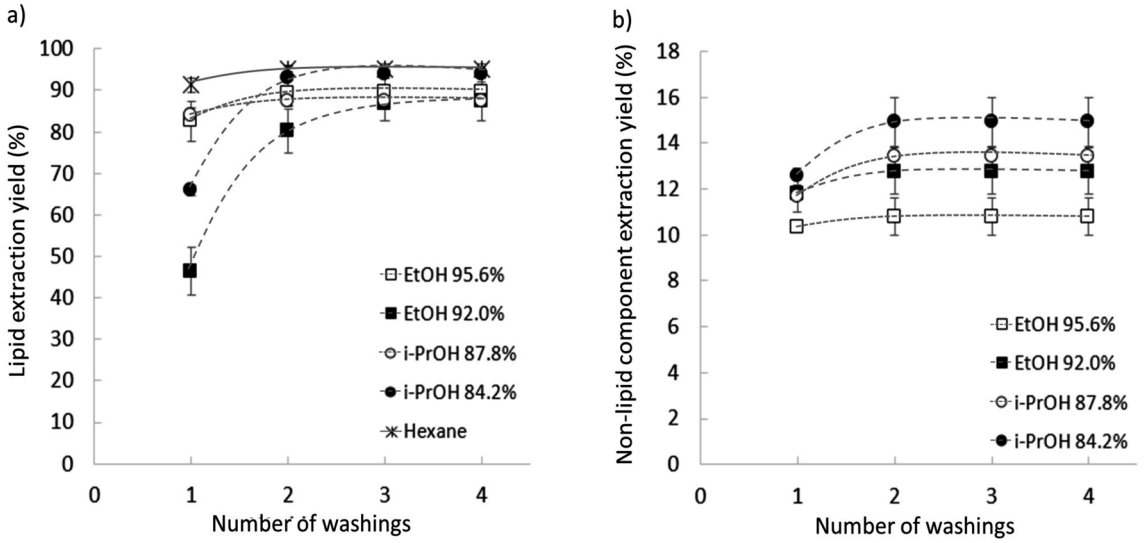

Fig. 2. Influence of solvent type and water content on a) lipid and b) non-lipid dry matter extraction yield in a 4-stage cross-current extraction. Error bars represent the standard deviation.

Scientific Inc., Saukville, WI) of total capacity 2.78 L, equipped with a double jacket for temperature control, an agitator for mechanical stirring, and a stainless steel 20 μm mesh (5 layers) on the bottom of the filter to separate the miscella from the solid after the desired extraction time. Four-stage cross-current extraction was performed by immersion of crushing seeds in preheated solvent (solvent/seed ratio: 1500 g/100 g per stage; 10 min per stage, stirring at 42 rpm and 50 °C). Miscella was recovered by filtration after each washing step, and the miscella samples were used to measure the extracted matter or to analyze the proportion of lipid and non-lipid fractions in the extracted dry matter. Finally, the meal was exposed to ambient air to remove the solvent.

Isopropanol at 84.2 wt.% or 87.8 wt.% alcohol, ethanol at 92.0 wt.% or 95.6 wt.% alcohol, and hexane (as reference) were tested as extraction solvents.

2.3 Analysis

2.3.1 Analytical measurements

Samples of miscella were desolventized in an oven (103.5 °C, 13 h) to measure dry matter content. Lipid and non-lipid contents of each miscella sample were determined by liquid-liquid extraction in hexane then water (1:1:0.4 wt/wt/wt miscella/hexane/water).

Total oil content of seeds and meals was determined according to NF v03-908. Total oil content of the meal is the sum of the extractable and non-extractable oil content. Extractable oil content was determined after Soxhlet extraction of the meal in hexane. Non-extractable oil content is the part of the residual oil content of the meal that is not readily extractable due to a seed grinding problem. It was quantified by a second grinding and extraction step run on the meal in hexane according to NF v03-908.

Moisture, protein and glucosinolate content of seeds and meals were determined according to ISO 771, ISO 5983-2 and ISO 10633-1, respectively. Protein solubility was determined by precipitation in potash according to ISO 14244. Protein and glucosinolate contents of seeds and meals were expressed on de-oiled dry matter basis using the following equation:

$$C_i^{DDM} = \frac{C_i^{DM}}{1 - C_L^{DM}}, \quad (1)$$

where C_i^{DDM} is concentration of component i (protein or glucosinolates) in the meal expressed on de-oiled dry matter basis (per mass of DDM), C_i^{DM} is the concentration of the component i expressed on a dry matter basis (per mass of DM) and determined by analysis, and C_L^{DM} is total oil content in the meal determined by analysis (g/100 g of DM).

2.3.2 Extraction yield

Cumulated lipid and non-lipid dry matter extraction yields (Y_k) were determined using the following equation:

$$Y_k = \frac{\sum(C_k^{mis} \times m^{mis})}{C_k^{seed} \times m^{seed}}, \quad (2)$$

where m^{seed} is mass of seeds (g), m^{mis} is mass of miscella recovered at each washing step (g), C_k^{seed} and C_k^{mis} are content of component k (lipids or non-lipid dry matter) in the seeds and the miscella (%), respectively.

2.3.3 Statistical analysis

Experiments and analyses were repeated twice. To evaluate the effect of extraction conditions, data was submitted to analysis of variance (ANOVA, in Excel) with $p < 0.05$.

3 Results

3.1 Crude oil extraction yield

Figure 2a reports lipid extraction yield after each washing step depending on type of solvent. The difference between

Table 2. Composition of meals after 4 washings (solvent/matter ratio: 15 g/g, temperature: 50 °C).

Sample	Oil content (g/100 g of DM)			Glucosinolate content (μmol/g DDM)	Protein content (g/100 g of DDM)	Protein solubility (%)
	Total oil content	Extractable oil content	Nonextractable oil content*			
Rapeseed	49.5	–	–	34	38.0	ND
Meal hexane	3.2	1.7	1.6	ND (34**)	37.6 ± 0.1	87
Meal 95.6 wt.% EtOH	3.6 ± 0.6	0.8 ± 0.2	2.8 ± 0.5	19	42.1 ± 0.1	77
Meal 92.0 wt.% EtOH	6.7 ± 1.7	2.2 ± 1.1	4.5 ± 0.7	14	42.9 ± 0.3	74
Meal 87.8 wt.% i-PrOH	5.3 ± 1.3	1.0 ± 0.2	4.3 ± 0.2	14	43.2 ± 0.2	68
Meal 84.2 wt.% i-PrOH	6.4 ± 1.6	1.0 ± 0.2	5.5 ± 1.4	7	43.6 ± 0.8	60

DM: dry matter basis; DDM: de-oiled dry matter basis; ND: not determined.
* Nonextractable oil content due to a seed grinding problem.
** Value estimated based on previous experiments.

solvents was mainly observed in the first wash: lipid solubility was reduced by the presence of water in alcohol, and hexane remained the most efficient solvent in terms of oil extraction. Lipid extraction yield thus reached 91% with hexane and 83–84% with azeotrope alcohols (95.6 wt.% EtOH and 87.8 wt.% i-PrOH) at the first extraction step. A 3.6 wt.% increase in water content reduced lipid extraction yield to 46% and 66% for 92 wt.% EtOH and 84.2 wt.% i-PrOH, respectively. Consequently, as oil solubility decreased with alcohol content, the process required a higher quantity of solvent, and so a larger number of washings was required in order to extract the same amount of oil. The negative impact of the water content in alcohol on oil solubility was previously demonstrated by Rao and Arnold (1956 and 1957) for both ethanol and isopropanol and by Sinichi and Diosady (2014) for isopropanol. Nevertheless, as shown in Figure 2a, final yield, *i.e.* (89.5 ± 3.7) wt.%, was not significantly impacted by the water content in the alcohol/water mixture ($p = 0.298$).

Furthermore, the variation in water content had less influence on lipid extraction by isopropanol than by ethanol. This result is in agreement with Rao and Arnold (1956, 1957) who demonstrated that the miscibility of vegetable oils was greater in isopropanol than in ethanol at a given water content.

Figure 2b reports non-lipid dry matter extraction yield. Alcohols extracted some non-lipid components as previously reported (Berot and Briffaud, 1983; Sinichi and Diosady, 2014). The extracted non-lipid dry matter represented 11 to 15 wt.% of the initial defatted matter depending on water content. As observed, the higher the water content, the higher the extraction of non-lipid components. The maximum values of non-lipid dry matter yield were achieved after two washing steps. Note that non-lipid components were not extracted with hexane.

After alcoholic extraction and conventional distillation of the miscella, the crude oil contained 75–87% lipids and 12–25% non-lipid compounds depending on solvent tested. These non-lipid compounds are impurities and will complicate oil recovery value. Recent results suggested that applying an oil recovery method by cooling the alcohol miscella instead of distillation leads to recovery of a non-lipid-compound-free oil, as the non-lipid compounds are retained in the solvent-rich phase (Oliveira *et al.*, 2012; Citeau *et al.*, 2018).

3.2 Rapeseed meal quality

Table 2 reports the composition of the meal after 4 extraction stages. Protein and glucosinolate concentrations were expressed on a de-oiled dry matter basis to compare results independently of the variation in residual oil content, particularly the oil content that was nonextractable due to a seed grinding problem.

The initial rapeseed contained 38 g of proteins per 100 g of de-oiled dry matter. The use of alcohol increased the protein concentration of the meal to around 42–44 g/100 g without significant difference between alcohol types and water content ($p = 0.087$) but significantly higher than after hexane extraction ($p = 1.64 \times 10^{-4}$). Thus, the extraction of additional non-lipid compounds (estimated as 11–15 wt.% of the initial defatted matter) increased meal protein concentration by 11–16% compared to the hexane extraction meal. Berot and Briffaud (1983) demonstrated that protein concentration could reach 60 wt.% after ethanol or isopropanol extraction when using de-hulled rapeseed. However, dehulling was not applied in the current study.

As shown in Table 2, alcohols removed glucosinolates from the meal, and the higher the water content, the lower the residual glucosinolates concentration, in agreement with Fauduet *et al.* (1995) and Van Megen (1983). Isopropanol 84.2 wt.% eliminated the most glucosinolates from the oilseed, decreasing glucosinolate concentration by 49–73% compared to meals extracted by the other alcohols.

Table 2 also shows that the use of alcohols reduced protein solubility. Note that the impact was greater with higher-water-content solvent, in agreement with Sawada *et al.* (2014) who demonstrated that the protein solubility of soybean was decreased by the presence of water in ethanol and the loss of solubility was accentuated by increasing temperature. A loss of solubility reflects changes in protein conformation and a partial denaturation of the protein that could impact its functionality.

4 Conclusion

This study investigated the influence of solvent type, *i.e.* ethanol and isopropanol, at two water concentrations, on rapeseed extraction efficiency and rapeseed meal quality. Alcohol-based solvents extracted 11–15 wt.% non-lipid

matters along with the oil, thus increasing protein concentration to 42–43 g per 100 g of meal and decreasing glucosinolates concentration to 7–19 g per 100 g of meal depending on type of alcohol. In comparison, protein and glucosinolate concentrations were 38 g and 25 g per 100 g of meal, respectively, after extraction with hexane.

Lipid extraction was less affected by the variation of water content in isopropanol than in ethanol. In the tested conditions, the increase of water content did not affect the final protein concentration of the meal, but strongly affected protein denaturation and removal of glucosinolates. In particular, isopropanol with the highest water content eliminated the most glucosinolates from the oilseed, decreasing glucosinolate concentration by 49–73% compared to meals extracted by the other alcohols.

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